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Synthetic biodegradable hydrogel delivery of demineralized bone matrix for bone augmentation in a rat model

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ABSTRACT

There exists a strong clinical need for a more capable and robust method to achieve bone augmentation, and a system with fine-tuned delivery of demineralized bone matrix (DBM) has the potential to meet that need. As such, the objective of the present study was to investigate a synthetic biodegradable hydrogel for the delivery of DBM for bone augmentation in a rat model. Oligo(poly(ethylene glycol) fumarate) (OPF) constructs were designed and fabricated by varying the content of rat-derived DBM particles (either 1:3, 1:1 or 3:1 DBM:OPF weight ratio on a dry basis) and using two DBM particle size ranges (50–150 or 150–250 μm). The physical properties of the constructs and the bioactivity of the DBM were evaluated. Selected formulations (1:1 and 3:1 with 50–150 μm DBM) were evaluated in vivo compared to an empty control to investigate the effect of DBM dose and construct properties on bone augmentation. Overall, 3:1 constructs with higher DBM content achieved the greatest volume of bone augmentation, exceeding 1:1 constructs and empty implants by 3- and 5-fold, respectively. As such, we have established that a synthetic, biodegradable hydrogel can function as a carrier for DBM, and that the volume of bone augmentation achieved by the constructs correlates directly to the DBM dose.

1. Introduction

Bone augmentation can be broadly defined as the formation of bone beyond the existing skeletal envelope at an orthotopic skeletal site. Bone augmentation may be attempted in the vertical or lateral direction in a variety of geometries and may be required for functional and/or aesthetic restoration. Inadequate bone volume may result from tooth extraction, disease, trauma, tumor resection, and other causes of deformities. Edentulous patients may require bone augmentation for implant placement, and disease causing tooth loss may interfere with the effectiveness of bone augmentation procedures [1].

Clinical indications for augmentation surgery include alveolar ridge, sinus or contour deficits. Alveolar ridge augmentation treats deficits of the three classes in the buccolingual and/or apicocoronal direction, which may involve shortage of width and/or height [1]. Sinus augmentation treats the atrophic posterior maxilla to enhance bone volume for implant placement [2]. Contour deficits are characterized by insufficient bone volume or projection at a skeletal site, which disrupts the expected facial contours [3]. Generally, autologous bone is the best grafting material; however, synthetic or other natural materials provide an alternative when autologous bone is unavailable, and off-the-shelf options greatly simplify the procedure [4]. To study bone augmentation in a preclinical animal model, it was essential to use an easily accessible region of compact bone with adequate space for surgery and implantation. As such, we designed a rat model with augmentation of the parietal bone in order to meet the above requirements, the parietal bone model having been established in the literature with clear success [5–8].

Interventions of the above sort frequently employ demineralized bone matrix (DBM), the acid-extracted organic matrix of bone. DBM functions as an osteoinductive and osteoconductive biomaterial, delivering osteogenic growth factors in a bioresorbable form [9]. Commercial DBM formulations often employ excipients, inactive substances primarily added to enhance the handling properties [3]. Biopolymer excipients lack significant tunability and suffer from irreproducibility compared to synthetic materials [10]. Using an excipient neglects the potential of the carrier phase to augment the osteogenic activity of the DBM. Some researchers have attempted to enhance DBM by adding growth factors including BMP-2 [11], VEGF [12] and TGF-β1 [13]; however, considerable

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interest and opportunity remains to explore the enhancement of any of these systems with the added flexibility of a synthetic drug delivery system.

Synthetic hydrogels such as oligo(poly(ethylene glycol) fumarate) (OPF) have the potential to enhance the osteogenic effect of DBM as injectable formulations for cell encapsulation and controlled drug release [14]. To that end, we investigated whether OPF, in a simple and unmodified form, could serve as a suitable delivery system for DBM. We hypothesized that, in a rat model of bone augmentation, greater bone volume and height would be achieved by OPF constructs with higher DBM content owing to the greater dose of osteogenic material and to accelerated degradation.

2. Materials and methods

2.1. Experimental design

The study was split into two parts. The first aimed at designing and testing the fabrication process, elucidating the influence of DBM content and particle size on the physical properties of the constructs, and testing the bioactivity of the DBM. The second part aimed at investigating the constructs in a rat bone augmentation model and evaluating gross appearance, augmented bone volume, maximum height, and overall tissue appearance by histology. The constructs formulated to complete the study design are listed in Table 1.

2.2. OPF synthesis

OPF was synthesized from poly(ethylene glycol) (PEG) (Sigma Aldrich, St Louis, MO) with a nominal number average molecular weight (M_n) of 3,350 Daltons (Da) as reported [14]. The resulting OPF had an M_n of 7500 ± 200 Da and a weight average molecular weight (M_w) of 36,300 ± 600 Da as determined by gel-permeation chromatography using a Waters system with a Styragel HR 4E column and chloroform solvent. The molecular weight was determined using a PEG standard curve.

2.3. DBM harvest and processing

DBM was harvested from the tibiae and femora of 12 week old male Fischer 344 rats based on reported methods [15,16] under the authorization of the Rice University Institutional Animal Care and Use Committee. Briefly, bilateral tibiae and femora were harvested and soft tissue was removed. The bone ends were removed and bone marrow was flushed with phosphate-buffered saline (PBS). Bones were crushed to yield bone fragments on the millimeter size scale. These fragments were placed in 95% ethanol at 4 °C for 16 h. The fragments were then moved to ethyl ether at 4 °C for 6 h. The bone fragments were filtered and dried. The fragments were further pulverized in a mortar and pestle and sieved to achieve fractions in the range of 50–150 and 150–250 μm. The DBM particle fractions were demineralized in 0.6 M HCl at 4 °C for 14 h then filtered and dried. DBM particles were stored at −20 °C.

Table 1
Composite hydrogel formulations investigated in the present study.

DBM:OPF[a]	Particle size (μm)	Media
In vitro		
1:3	50–150/150–250	PBS/Col PBS[b]
1:1	50–150/150–250	PBS/Col PBS
3:1	50–150/150–250	PBS
	DBM:OPF[a]	Particle size (μm)
In vivo		
1:1	1:1	50–150
3:1	3:1	50–150
Empty	N/A	N/A

[a] DBM:OPF refers to the ratio of the components on a dry weight basis.
[b] Col PBS refers to PBS supplemented with 400 ng/ml collagenase 1A.

2.4. Composite fabrication

In the present study, our delivery system was produced by mixing OPF hydrogel precursors with DBM particles and cross-linking the OPF creating a hydrogel composite. Composites were fabricated at a size matching the implants for the animal experiment (8 mm diameter × 1 mm height) using an established method [17] as follows. Dry components, OPF, PEG-diacrylate (Glycosan BioSystems, Inc., Alameda, CA) and DBM, were combined and added to PBS. Thermal radical initiators, ammonium persulfate and tetramethylethylenediamine (Sigma Aldrich, St Louis, MO), were added to initiate crosslinking and the mixture was injected in a Teflon mold and incubated at 37 °C for 8 min for crosslinking to proceed. After 8 min, the composite hydrogels were removed from the mold and cultured in vitro or implanted in the rat. For the in vitro study, constructs were placed in 24 well plates in 1 ml PBS or PBS supplemented with 400 ng ml^{-1} collagenase 1A (Sigma Aldrich, St Louis, MO) (Col PBS) to mimic the level of enzymatic digestion that would occur in vivo [18]. Composites were maintained at 37 °C on a shaker table at 70 rpm with medium changes after 1 day and continuing twice weekly.

2.5. In vitro histology

Histology of in vitro samples was performed by embedding composite hydrogels in Histoprep freezing media and making 10 μm frozen sections using a cryotome (Leica 1850CM UV). Sections were stained with Picrosirius Red to reveal the collagen matrix of the DBM particles. Images were taken using a light microscope (Nikon Eclipse E600).

2.6. Physical characterization

The composite hydrogels in the present study were characterized in terms of their degradation, mass swelling and compressive mechanical properties according to reported methods [18]. Degradation or normalized mass was measured as the fraction of dry mass remaining at day 1 and at 1, 3 and 5 weeks relative to the dry mass at day 0 ($n = 3$ samples). Mass swelling was measured as the ratio of wet weight to dry weight of the samples at each time point at day 1 and at 1, 3 and 5 weeks ($n = 3$). Compressive mechanical properties were measured at 1, 3 and 5 weeks by thermomechanical analysis (TMA 2940, TA Instruments, New Castle, DE) at a compression rate of 0.1 N min^{-1} where the compressive modulus was determined as the initial slope of the stress–strain curve ($n = 3$).

2.7. Osteogenesis assessment

The relative osteogenic activity of DBM was determined using the C2C12 cell line assay (ATCC) [19]. C2C12 cells were expanded in high-glucose Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (BenchMark™ FBS, Gemini Bio-Products, West Sacramento, CA) for several days. C2C12 cells were lifted with 0.25% trypsin and seeded at 50,000 cells per well in a 24 well culture plate and allowed to attach overnight ($n = 5$). Medium was replaced with high-glucose DMEM with 1% FBS for the control or supplemented with 4 mg ml^{-1} DBM (based on previous experiments that investigated this concentration [19]) with particle sizes of 50–150 μm for the test. Alkaline phosphatase (ALP) activity (nmol *p*-nitrophenol h^{-1}) was determined and normalized to DNA (μg).

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